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The Agricultural Chemical Society of Japan.

President : Keijiro Aso.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

STUDIES ON THE SPECIFIC PROPERTIES OF PROTEASE AND AMYLASE FROM THE STANDPOINT OF THE ADSORPTION PHENOMENA.

I. COMPARATIVE RELATION BETWEEN TAKA-DIASTASE AND PANCREATIN.

By

Fumiwo Hemmi and Goro Inami.

(From the Research Laboratory of the Agricultural Technology, College of Agriculture, Hokkaido Imperial University.)

(Received May 24th., 1929)

It is probably to say that the recent progress of the enzyme chemistry is due to the application of the method of adsorption, especially for the purification of enzyme. The authors were obliged to clear the specific properties of enzymes from Aspergillus oryzae and other fungi, when we have studied on the relation of the enzymic action for the manufacture of "Soy-sauce." The present investigation was done to clear the specific properties of enzyme from the standpoint of adsorption and also of other physico-chemical properties, because it was conjectured to exist an important and delicate difference among the specific properties of enzymes with the origin of animals, plants and fungi.

It is one part of our studies to report here; that is a comparative research between Taka-diastase and pancreatin, the former of which was made from the culture of Aspergillus oryzae and the latter from an animal organ, pancreas.

The conclusion obtained from the results of our experiments are as follows :-

1) Free amino acid and reducing sugar, which contained in the enzyme solution, are probably adsorbed by neither aluminium hydroxide nor kaolin.

2) Protease and amylase of Taka-diastase were easily adsorbed by aluminium hydroxide from their aqueous solution in the amount of two and four times than those of pancreatin for the same quantity of adsorbent used. On the contrary, panc.-protease was always adsorbed by kaolin more than two times in amount compared with that of Taka-protease for the same quantity of adsorbent used. Taka-amylase was not practically adsorbed by kaolin, but panc.-amylase was adsorbed by kaolin about 35% of the initial

enzymic power, when kaolin corresponding to nearly thirteen times of the quantity of pancreatin was used (Fig. 1–4).

3) The adsorption degree of Taka-protease by aluminium hydroxide was rapidly decreased, according to the increasing of PH value from 4.4 to 7.0, and at the weak alkaline solution above PH=7.0, no adsorption of Taka-protease was observed, while panc.-protease under the same condition was adsorbed in a small amount (Fig. 5).

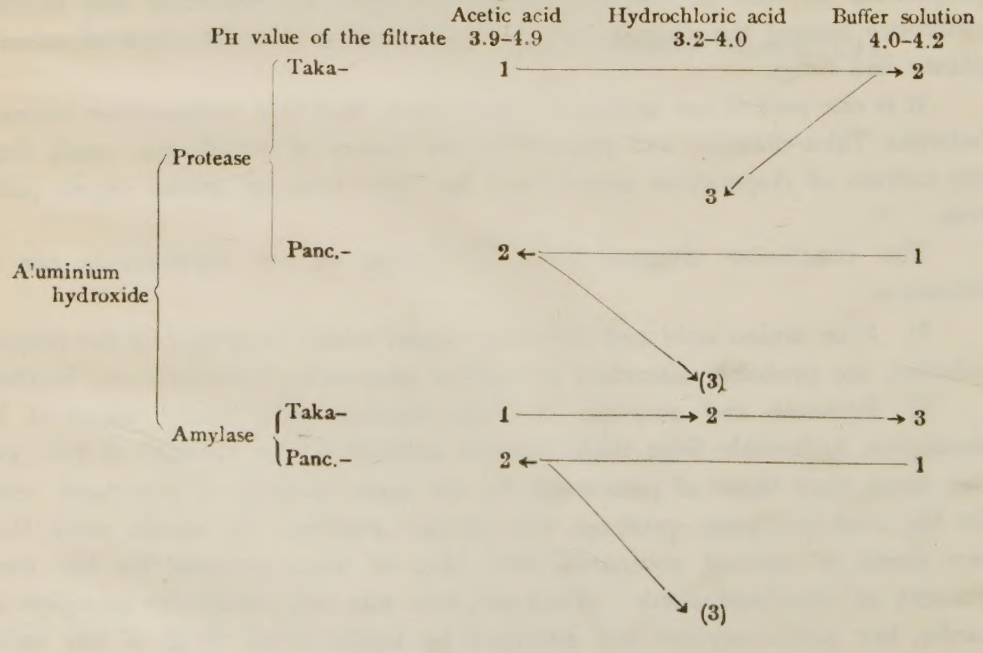
Taka-amylase was rather decreased its adsorption degree for aluminium hydroxide by the addition of phosphate buffer solution by McIlvain, PH=3.8-8.3, compared with that of no addition of buffer mixture, but on the contrary the adsorption of panc.-amylase was remarkably increased (Fig. 6).

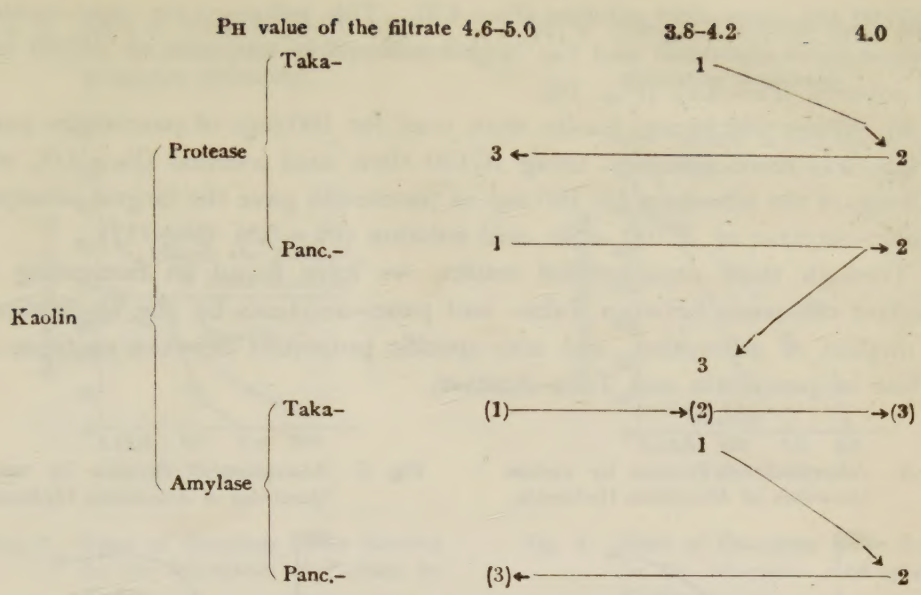
4) The adsorption degree of Taka-protease by kaolin was increased by the addition of buffer solution, PH=3.8-8.3, but in the case of panc.-protease, the decrease of adsorption was observed (Fig. 7).

It will be an interesting matter that panc.-amylase was more easily adsorbed by kaolin with the addition of buffer solution, but the increase of adsorption for Taka-amylase under the same condition was always small when compared with panc.-amylase (Fig. 8).

5) The effects of acetic and hydrochloric acids and phosphate buffer solution on the adsorption of protease or amylase from an aqueous solution of Taka-diastase or pancreatin were also studied. The concentration of added acid to the total solution was $N/100$ or $N/200$ for aluminium hydroxide adsorption and $N/250$ for kaolin adsorption. The PH value of the buffer solution used was 4.0.

The results obtained were briefly given in the next table.





( ) very little adsorbed.

6) Protease of Taka–diastase and pancratin was well adsorbed by kaolin in any case with the addition of hydrochloric, sulphuric, acetic, lactic, citric, tartaric and oxalic acids. But the adsorption of Taka–amylase by kaolin was very poor, in any case using acids above mentioned, of which, in good case, 15% adsorption of enzyme was obtained in sulphuric acid solution, while the adsorption of panc.–amylase by kaolin was 94–98% for hydrochloric, tartaric, sulphuric, lactic, citric, and oxalic acids and 80% for acetic acid.

The specific properties of the enzymes between Taka–diastase and pancreatin were easily distinguished by the adsorption of kaolin. And also in the case of the adsorption of aluminium hydroxide, the special difference between them was observed.

7) In the case of aluminium hydroxide adsorption, Taka–protease (in acid solution with lactic acid) and panc.–protease (in acid solution with citric acid) were adsorptive, when the acid concentration of adsorption medium varies from $N/200$ (P_H values of each mixture were 4.8 and 4.3 respectively) to $N/150$ (P_H values of each mixture were 4.3 and 3.9 respectively). The adsorption degree of amylase (Taka and panc.–) was the largest when each mixture was $N/200$ solution, P_H values of which were 4.8 and 4.3 respectively, and this phenomenon had no relation to the quantity of aluminium hydro xide at some extent (Fig. 9).

8) In the case of the adsorption by kaolin, Taka–protease was remark ably influenced by acid concentration, for instance 14.3% of Taka–protease were adsorbed at $N/400$ lactic acid solution ($P_H=5.1$), while 94.8% of that

at $N/150$ the same acid solution ($P_H = 4.3$). This influence for panc.–protease was comparatively small and the largest adsorption was seen at $N/200$ citric acid solution ($P_H = 4.1$) (Fig. 10).

9) When 335.25 mg. kaolin were used for 100 mg. of pancreatin, panc.–amylase was more adsorbed, using $N/150$ citric acid solution ($P_H = 4.0$), while 670.5 mg. of the adsorbent for 100 mg. of pancreatin gave the largest adsorption of panc.–amylase at $N/100$ citric acid solution ($P_H = 3.8$) (Fig. 11).

Through these experimental results, we have found an interesting and important difference between Taka– and panc.–amylases by the application of the method of adsorption, and also specific properties between protease and amylase of pancreatin and Taka–diastase.

Fig. 1. Adsorption of Protease by various Quantities of Aluminium Hydroxide.

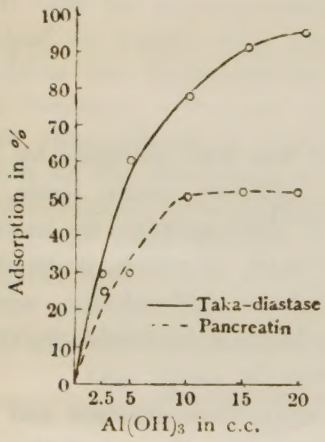


Fig. 2. Adsorption of Amylase by various Quantities of Aluminium Hydroxide.

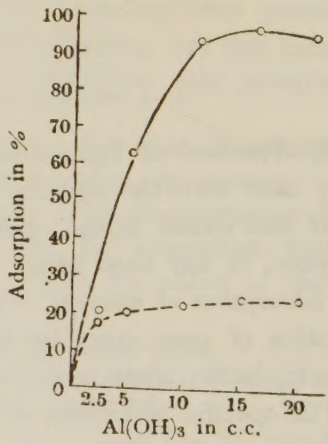


Fig. 3. Adsorption of Protease by various Quantities of Kaolin.

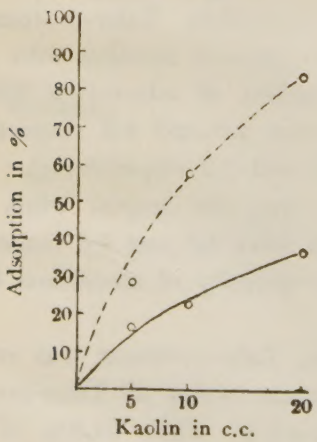


Fig. 4. Adsorption of Amylase by various Quantities of Kaolin.

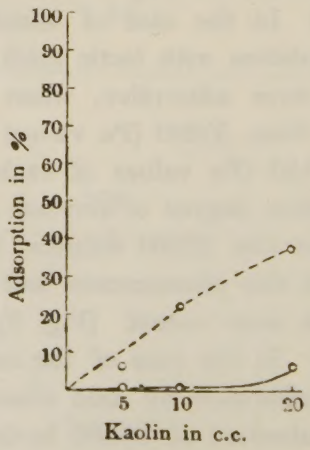


Fig. 5. Effect of Phosphate Buffer Solution for the Adsorption of Protease by Aluminium Hydroxide.

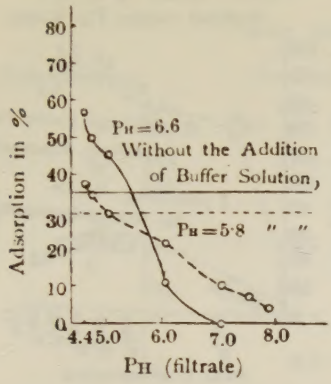


Fig. 6. Effect of Phosphate Buffer Solution for the Adsorption of Amylase by Aluminium Hydroxide.

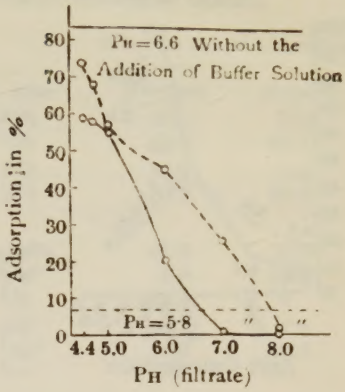


Fig. 7. Effect of Phosphate Buffer Solution for the Adsorption of Protease by Kaolin.

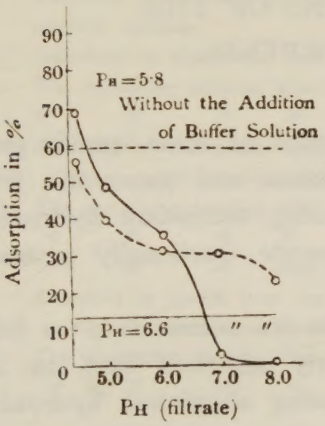


Fig. 8. Effect of Phosphate Buffer Solution for the Adsorption of Amylase by Kaolin.

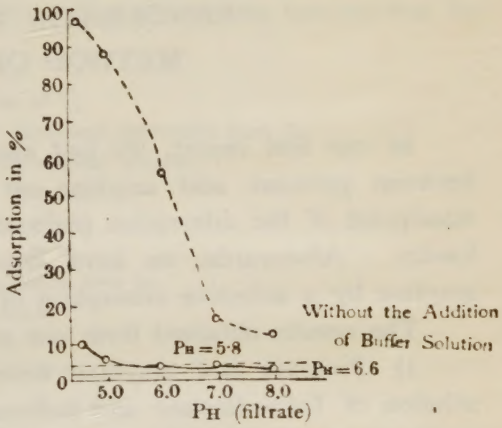
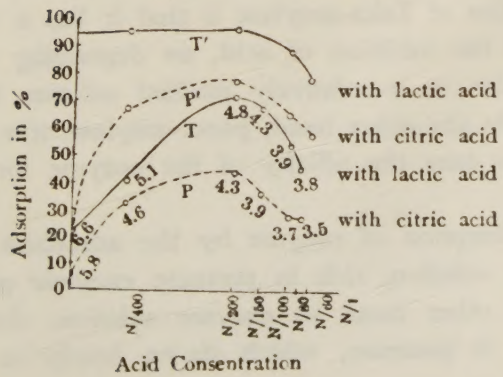


Fig. 9. Relation between the Adsorption of Amylase by Aluminium Hydroxide and the Concentration of added Acid (and PH Value).



Number means PH Value.
The quantity of adsorbent used for P′ and T′ was double of those for P and T respectively.
P, P′ Pancreatin
T, T′ Taka-diastase.

Fig. 10. Relation between the Adsorption of Protease by Kaolin and the Concentration of added Acid (and PH Value) Number means PH Value.

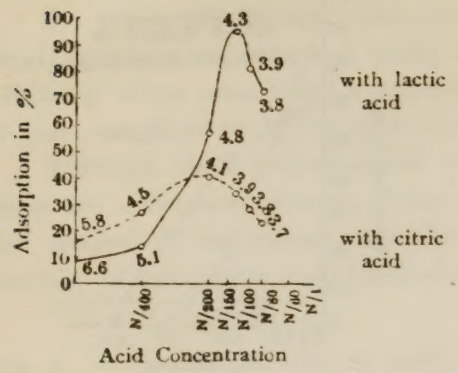
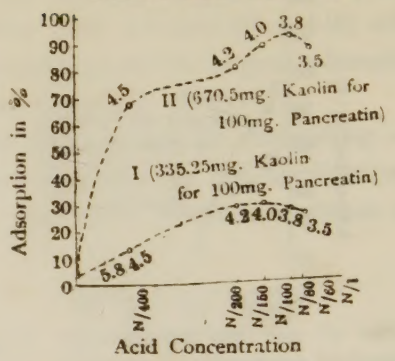


Fig. 11. Relation among the Quantity of Adsorbent, Acid Concentration used and PH value for the Adsorption of Panc.-amylase by Kaolin. Number means PH Value.



II. THE SELECTIVE SEPARATION OF PROTEASE AND AMYLASE FROM TAKA-DIASTASE AND PANCREATIN BY MEANS OF THE METHOD OF ADSORPTION.

In our first report, we had already reported on some specific properties between protease and amylase of Taka-diastase and pancreatin from the standpoint of the adsorption phenomena by using aluminium hydroxide and kaolin. Afterwards, we have tried to separate fractionally protease and amylase by a selective adsorption of enzyme.

The results obtained from our experiments are summarized as follows :-

1) Protease and amylase were selectively adsorbed from an aqueous solution of Taka-diastase and pancreatin by using aluminium hydroxide and kaolin as adsorbents. But when an enzyme solution, acidified by the addition of acid, was used, a selective adsorption was not, in many cases, observed.

2) One of the special properties of Taka-amylase is that it has a weak affinity for kaolin, with or without the addition of acid, no depending upon the quantity of kaolin used and also in a relatively purified solution freed from most parts of impurities. On the other hand, panc.-amylase was well adsorbed by kaolin, because in this case the affinity of the enzyme for the adsorbent was strong.

3) Applying the selective adsorption of enzyme by the adsorbent, we had succeeded to get an enzyme solution, rich in protease enzymic power but poor in amylase, and on the other hand an enzyme solution, rich in amylase enzymic power but poor in protease, which shown briefly in the

next figures.

Enzymic power determined after applied the method of the adsorption, using the buffer mixture of $P_H=4.4$ (As an enzymic power of the original solution $=100$)

		After the first adsorption.			After the second adsorption.	
Taka-diastase		Protease	Amylase		Protease	Amylase
I.	A_1	68.3	10.5	A_2	66.1	6.0
	B_1	6.0	73.4	B_2	1.6	61.7
II.	C_1	41.4	10.0	C_2	16.1	1.1
	D_1	4.8	67.8	D_2	0.0	46.8
Pancreatin						
I.	a_1	64.3	81.7	a_2'	41.5	0.0
				a_2''	2.6	63.9
	b_1	35.7	6.3	b_2	11.1	0
II.	c_1	52.6	87.4	c_2'	46.7	2.5
				c_2''	1.3	65.8
	d_1	9.2	0.0	—	—	—

A_1 : Adsorbed by kaolin from the original solution.

A_2 : 〃 again by kaolin from A_1.

B_1 : 〃 by aluminium hydroxide from the remaining solution after the separation of A_1.

B_2 : The remaining solution after the removal of adsorbate by kaolin from B_1.

C_1 : Adsorbed by kaolin from the elution of the adsorbate by aluminium hydroxide from the original solution.

C_2 : Adsorbed by aluminium hydroxide from C_1.

D_1 : The remaining solution after the separation of C_1.

D_2 : 〃 〃 〃 〃 adsorbed by aluminium hydroxide from D_1.

a_1 : Adsorbed by aluminium hydroxide from the original solution.

a_2' : 〃 again by aluminium hydroxide from a_1.

a_2'': The remaining solution after the separation of a_2'.

b_1 : 〃 〃 〃 〃 〃 〃 〃 a_1.

b_2 : 〃 〃 〃 after adsorbed by kaolin from b_1.

c_1 : Adsorbed by koalin from the original solution.

c_2' : 〃 by aluminium hydroxide from c_1.

c_2'': The remaining solution after the separation of c_2'.

d_1 : 〃 〃 〃 〃 〃 〃 〃 c_1.

(Sapporo, Japan.)

BIOCHEMICAL STUDIES OF *SALMONIDAE*. I.

ON THE METABOLIC CHANGES THROUGH THE EGG AND LARVAL STAGES OF THE SALMON [*ONCORRHYNCHUS KETA* (WALBAUM)]

By

HIDESABURO SEKINE.

(Received Oct. 7 th., 1929)

The samples for this study, the eggs of which had been transported from The Murakami Hatchery, Niigata Prefecture, are cultured in a glass vessel in the laboratory, until they hatched out and absorbed their york-sacs. In the course of the culture, the next three samples are taken for the analyses.

1. Eggs are 52 days old, on January 8th.
2. Larvae are just hatched out on the last days of February.
3. Young salmons entirely absorbed their york-sacs.

The results of the analyses of these samples are summarized as follows:-

	Live wt.	Water	Solids	Inorg. matters	Lipins	Proteins P.N × 625	T. N.	Protein N.	Alcohol soluble N.	Alcohol soluble P.
1.	253.50	156.41	97.09	3.11	28.69	74.46	12.97	11.91	1.33	0.34
2.	234.40	141.59	92.81	4.04	23.17	69.58	11.65	11.13	1.32	0.54
3.	319.44	259.04	60.40	5.21	11.34	36.40	6.31	5.83	0.71	—
1.	100.00	61.70	38.30	1.23	11.32	29.37	5.108	4.699	0.53	0.13
2.	100.00	60.40	39.60	1.37	9.89	29.70	4.974	4.749	0.56	0.23
3.	100.00	81.09	18.91	1.63	3.55	11.40	1.975	1.820	0.22	—
1.			100.00	3.20	29.55	76.68	13.36	12.27	1.37	0.35
2.			100.00	4.35	24.97	74.99	12.56	11.99	1.42	0.58
3.			100.00	8.62	18.38	60.27	10.44	9.64	1.22	—

Summary

The eggs (52days old) lose of water and solids until they hatch out, but there are increasion of inorganic matters and alcohol soluble phosphorus (lecithine like substance) and decreasion of proteins and lipins.

Some amounts of these decreased substances come probably from the constituents of the egg's membrance and the substances of its perivitellin space both of which won out from the larva, but the absorption of the mineral matters and the formation of some phospholipins are admitted as noticable phenomena.

In the next time, there is a considarable metabolic change through larval stage. A youg salmon just absorbed its york-sac gains 85 mg. in weight as the result on absorbing 117 mg. of water and consuming 35 mg. of solids.

At any rate, the growth phenomenon through egg and larval stages are consumption of the greater part of the organic matters in them and the assimilation of some mineral matters and large amount of water from the surrounding medium, because several newly constituents as phospholipins or proteins of muscles and etc., are synthesized from the materials contain originally in this organism.

BIOCHEMICAL STUDIES ON *SALMONIDAE* II.

ON THE DEFECTS OF SILKWORM'S PUPAE AS FOOD OF YOUNG TROUTS (*SALMO IRIDEUS*.)

By

Hidesaburo Sekine, Minoru Kawajiri and Yuji Kakizaki.

(Received Oct. 7th, 1929)

This paper reports the study on the nutritive value of silkworm's pupae which utilized largely as food for feeding fishes in this country.

The feeding experiments were carried out for 63 days, from July 28 th., to September 28 th., 1927, at The Fish Culture Station by the Lake of Kisaki, Nagano Prefecture.

The experimental animals—young rainbow trouts 180 mg. in weight—were divided into 3 groups each of which has 300 fishes. The first group were fed on a basal ration which consisted principally powdered pupa mixing starch of 20%. The second group were supplied the above ration with addition of Osborne's salt mixture and some raw fish, and the diet for the third was that which was added the salt mixture and a small amount of cod liver oil, rice bran and a juice of radish as sources of vitamins A, B and C on the basal one.

The results of the feeding experiments:– All of them are summarized in the Table I and II.

Table I.

Number of fishes	Group I		Group II		Group III	
Opening	300	%	300	%	300	%
Ending	19	6.3	14	4.7	115	38.3
Loss	281	93.7	286	95.3	185	61.7
Dead	198	64.3	282	94.0	157	52 3
Missing	88	29.3	4	1.3	28	9.3

Table II. The total weights of the fishes in each group on the opening and on the ending of the experiments.

	Group I	Group II	Group III
Opening (g.)	54	52	56
Ending	25	17	80
Per cent	46	33	143

In view of these results, the survived animals in the first group were only 6 % in number and 46 % in weight, and 70 % of the loss in the former case were dead while the others were missing. The remainders in the second group were 5 % and 33 %, and the majorities (90 % in number) of which died away at early period of the feeding and the missings, therefore, were very few. But 40 % of the third group were survived and gained 43 % in weight. This good figures in the last case should probablely resulted by the addition of the vitamins.

The analytical results :– The results of chemical analysis of the survived animals in each group show in the Table III and IV.

Table III. Individual chemical composition of 3 samples in each group.

		Live weight	Water	Solids	Inorg. matter	Organic matter	Lipins	Nitrogen
Group I	A	1540	1205	335	23.3	311.6	76.6	37.74
		100	79.25	21.75	1.52	20.23	4.97	2.451
				100.00	6.97	93.03	22.88	11.27
"	B	1310	1023	287	17.9	269.0	67.1	31.57
		100	78.09	21.91	1.37	20.54	5.12	2.41
				100.00	6.24	93.76	23.38	11.10
"	C	740	597.5	142.5	8.08	134.4	27.2	15.9
		100	80.77	19.23	1.09	18.14	3.68	2.149
				100.00	5.68	94.32	19.12	11.18
Group II	D	1400	1082	318	12.01	306.0	67.4	35.62
		100	77.25	22.72	1.91	20.31	4.82	2.559
				100.00	8.55	91.45	21.19	10.52
"	E	1030	870	163	9.36	153.6	19.1	19.36
		100	83.25	16.75	1.52	15.23	1.85	18.81
				100.00	9.09	90.91	11.38	11.23
"	F	750	586	164	6.20	158.8	40.0	18.01
		100	78.09	21.91	1.81	20.10	5.33	2.402

				100.00	8.27	91.73	24.35	10.96
Group III	G	1420	1132	288	21.0	267.0	61.1	27.83
		100	79.72	20.28	1.48	18.80	4.31	2.199
				100.00	7.30	92.70	21.26	10.84
"	II	1330	1040	290	22.67	267.3	63.5	31.73
		100	78.16	21.84	1.70	20.14	4.77	2.385
				100.00	7.80	92.20	22.38	10.92
"	I	1040	829	221	18.39	202.6	44.4	21.01
		100	79.68	20.32	1.77	18.55	4.27	2.036
				100.00	8.70	91.30	21.01	10.02

Table IV. Average compositions of the remainders in each group.

	Solids	Inorg. matter	Organic matter	Lipins	Nitrogen
Group I	201.9	5.97	94.03	20.78	10.75
Group II	197.2	8.12	91.88	19.68	10.65
GroupIII	214.6	7.97	92.03	21.95	10.50

From these results, compositions of animals which fed on the same diet are not always equal but inorganic contents of the animals in the first group are surely less than those in the others. It may be probably seen that the difference comes from whether the salt mixture added to the basal diet or not.

Furthermore, the chemical composition of rainbow troute just absorbed their york–sacs (hatched out in the Atkin's method) at the Lake of Numazawa are as follows :–

Table V.

Live weight	Solids	Inorganic matter	Organic matter	Lipins	Nitrogen
150.0	26.67	1.85	24.82	3.87	3.255
100.0	17.68	1.23	16.46	2.58	2.17
	100.0	6.94	95.06	14.57	12.30

Comparing the animals employed for the experiments with the above sample, those in the first group of the former are poorer but the others are richer in the inorganic contents of dry matter per cent than the latter because the diet of the first group contains no salt mixture and the others contain it. The basal diet, therefore, should be lacked in this constituents. And we may be pointed out that the experimented animals are, in generally, poorer in proteins and richcher in lipins than the original ones. From these facts, it should be assumed that the protein of the dry pupa is poor in nutrition and its lipins are surplus for want of fishes.

Conclusion

Silkworm's pupa lacks in inorgnic and vitamins contents as a food of young trouts and their protein show low value in nutrition and their lipins contents are surplus for want of the fishes.

The missings in feeding animals which fed on a food lacking in vitamins like dry silkworm's pupae should be get rid off by means of adding these factors.

BIOCHEMICAL STUDIES ON *SALMONIDAE* III.

ON THE NUTRITIVE VALUES OF FOODS MADE FROM FISHES.

By

HIDESABURO SEKINE assisted by YUJI KAKIZAKI.

(Received Jan. 6 th., 1930).

The feeding experiments carried out for 38 days, from May 24th. to June 30 th., in the hatching boxes through which containing always about 40 litres of running spring water, at The Kamaishi Hatchery in Iwate Prefecture.

The eggs of the experimental animals Oncorhyncus Keta (Walbaum) employed fertilized on December 27 th., 1926, hatched out on about February 29 th., 1927, and absorbed their york-sacs on May 24 th.

1500 young salmons were divided into 5 groups which were fed on the following diets :—

The first group ; no feed ; as blank.

The second group ; pasted, boiled rice polished.

The third group ; boiled, pressed and dried fish except its meat.

The fourth group ; boiled, pressed and dried fish.

The fifth groups ; unboiled fish, except its head and inner organs.

The date from the experiments are summarized as follows :—

TABLE I.

Group	I	II	III	IV	V
Total live weight and number.					
Initial live wts(g.) & numbers.	92.25 300	90.75 300	97.35 300	97.35 300	97.52 300
Live wts & numbers on the end.	34.50 121	19.13 63	80.25 154	166.88 186	313.50 292
Percentage	37.22 40	21.08 23	85.63 51	171.4 62	321.5 973
Percentages of group II—V to group I	100	55.4	232.6	486.0	908.9
Values per fish.					
Initial live weight (mg.)	308.8	302.5	312.5	324.5	325.1
Live weight on the end.	285.1	281.2	521.1	824.5	1074.0
Percentage	92.34	92.96	170.4	276.5	333.6
Percentages of group II—V to group I.	100	98.63	186.8	314.7	376.8

Values calculated as the same weight at the initial weight of all groups.

Percentages of group II—V to group I					
Total number	100	57.5	127.5	155	243.3
Total weight	100	56.6	230	460.5	863.8
Per fish	100	100.7	163	297.3	361.3

According to Bodine[1] starvation in the insect grasshopper on the land results in a rapid loss in water content, which has a decided quick and fatal effect. But on the same condition of the fish in the water shows no decrease in this content and it seems as the longer life is there, because it is very doubted that there is no food material except inorganic matter in the medium although feed on nothing.

From the above date, 40% of the fishes in the group I survived for 38 days fasting and their water contents increased in spite of its decrease which usually brings to fasting animals on the land.

On the values of the fed materials, polished rice shows not only the lowest value but poorer than foodless. Unboiled fish shows the heighest value and the next is dried fish and the third dried fish except its meat.

Then the analytical results of the experimented animals and the young salmons just absorbed their york-sacs which employed for these experiments are shown in the Table II.

TABLE II.

	Live weight	Dry matter	Inorganic matter	Organic matter	Lipins	N	Lipins–N	Ca	Mg	P
Young salmons just absorbed their york-sacs	315.0	53.33	2.438	50.89	13.61	6.034	——	0.4206	0.1288	0.6032
	100.0	16.93	0.774	16.156	4.32	1.917	——	0.1335	0.041	0.1915
		100.00	4.569	95.431	25.50	11.32	——	0.788	0.245	1.131
I	285.1	43.14	4.796	38.34	8.13	4.922	0.0814	0.959	0.0414	0.683
	100 0	15.13	1.682	13.45	2.87	1.727	0.0286	0.3365	0.0145	0.2395
		100.00	11.137	88.86	18.97	11.412	0.1887	2.224	0·0961	1.5834
II	281.2	44.2	4.503	39.70	7.51	4.834	0.0789	0.881	0.0512	0.965
	100.0	15.72	1.602	14.12	2.67	1.719	0.0280	0.313	0.0182	0.279
		100.00	10.193	89.807	16.99	10.924	0.1784	1.993	0.1158	1.771
III	521.1	91.6	12.30	79.30	13.83	11.19	0.1602	1.377	0.1442	1.485
	100.0	17.58	2.365	15.22	2·66	2.195	0.0306	0.264	0.0277	0.285
		100.00	10.668	89.33	15.10	12.464	0.1738	1.520	0.1574	1 639
IV	897.2	147.45	15.20	135.2	23.33	16.25	0.2423	2.537	0.2834	2.815
	100.0	16.44	1.693	14.75	2.60	1.81	0.0270	0.283	0.0316	0.311
		100.00	10.307	89.69	15.45	11 016	0.1643	1.721	0.1923	1.909
V	1074.0	212.86	16.89	196.0	38.52	21.95	0.5008	2.577	0.3816	3.305
	100.0	19.81	1.571	18.24	3.59	2.04	0.0435	0.240	0.0352	0.308
		100.00	7.934	92.07	18.10	10.315	0.2197	1.211	0.1793	1.553

From this figures in the Table II, percentageous values of all the constitutions of the experimented animals to those on opening of these experiments are caluculated as follows :–

TABLE III.

	Live weight	Dry matter	Inorganic matter	Organic matter	Lipins	Nitrogen	Ca	Mg	P
I	0.91	0.81	2.0	0.75	0.60	0.82	2.3	0.32	1.1
II	0.90	0.83	1.9	0.78	0.55	0.80	2.1	0.40	1.6
III	1.65	1.7	5.0	0.13	1.2	1.9	3.3	1.1	2.5
IV	2.85	2.8	6.2	2.7	1.7	2.7	6.0	2.2	4.5
V	3.41	4.0	6.9	3.8	2.8	3.6	6.1	3.0	5.5

Sergius Morgulis[2] reported that brook trouts loss about one-fifth of their body weight during 28 days fasting.

On 38 days fasting, in this experiment, young salmons lose about 10 % in their live weights, 20% of solids and 25% of organic matter that is 40% of lipins, 18 % of proteins, but inorganic matter is doubled, on the contrary, Ca and P are 230%, 110% respectively, but Mg decreases 68%. Therefore, metabolisms of Ca, P and Mg on fasting are carried out in an opposit direction. It is probablly that the fish assimilates Ca and P always from the water notwithstanding on fasting whilst Mg is excreted from the body along to the protein catabolism.

The fishes were fed on boiled rice are poorer than the foodless, and it seems to that this food has not only nutritive value but increasing catabolic changes of proteins and lipins. The fishes were fed on dried fish except its flesh increased only 7 % in solids and on dried fish and fresh one become 3.5 and times in solids respectively.

On the percentage of live weights of experimental animals, comparing fed ones with that of the fish before experiment, the fishes in group I and II poorer in their solids but richer in water content, and the others (III, IV and V) increase their solids. Organic matter decreases as the result of the increase of inorganic matter and lipins decrease also but proteins (N) increase.

In view of the percentage of solids, it is very curious that it may be not found almost any differences among the fishes in all groups notwithstanding they show very different degrees in growth.

Conclusion.

1. On fasting, there occur consumptions of organic matters in solids and accumulations of inorganic matter, on the contrary, especially Ca and P greatly increase but Mg decreases along to the proteins metabolism.

2. Polished rice which consisted with almost pure starch has not only nutrive value but it accerelates the exclusions of proteins and lipins.

3. Boiled, pressed and dried fish with out meat, the same one with meat and the fresh except its head and inner organs show very different nutritive values in the points of decreasion in numbers, total and average weight of

fishes. The first is the poorest in them and its lipins and Mg contents are not increased for 38 days feeding, while the one fed on fresh becomes about 4 times in organic and 7 times in inorganic, and the second group shows the value between them.

4. Comparision of the nutritive values of food for fishes can be decided only in the actual weights in fresh body, and percentageous values of them can not be applied for these purposes because little difference among them are expressed.

Literature.

(1) Bordine: J. Exp. Zool., 32; Shelford; Laboratory and field Ecology. P. 155.
(2) Sergius Morgulis: J. Bio. Chem, XXXVI. 39, (1918).

BIOCHEMICAL STUDIES ON *SALMONIDAE* IV.

ON AN EFFECT OF THE LIGHT ON THE METABOLISM OF THE FASTING YOUNG SALMON [*ONCORHYNCUS KETA* (WALBAUM)]

By

Hidesaburo Sekine assisted by Yuji Kakizaki.

(Received Jan. 6th., 1930).

According to the preceeding paper, bodily comsumption of young salmons for 38 days fasting is about 10 % in their live weights, solids and lipins are 20 % and 40 % respectively, while inorganic content is doubled, on the contrary, by assimilation from the surrounding medium.

This paper reported the further study was carried out on an effect of the light during fasting.

Salmon's eggs were transported from The Chitosé Hatchery in Hokkaido, and then hatched out in a hatching-box (in the darkness) in our laboratory, some of them, as they entirely absorbed their york-sacs, were transported in a glass vessel (in the light) by the window. After 14 days fasting, none of them died in the light as if their appearances were entirely starving whilst the majorities of the other died away in the darkness.

The two samples in light and dark on the end of these experiments were analysed and compared with that of the sample had been taken at just absorbed their york-sacs.

TABLE I.

	Stage at york-sacs are just absorbed	14 days fasting in the light	14 days fasting in the darkness
Number of fishes	50	30	50
Total live weight	10.8296	5.7018	10.4290
Total solids	2.3593	1.0731	1.7156

Their actual contents and percentageous amounts in the constituents are as follows :—

TABLE II.

	Stage at york-sacs are just absorbed			14 days fasting in the light			14 days fasting in the darkness		
Live weight	216.5	100		190.1	100		208.1	100	
Water	169.3	78.21		154.3	81.18		174.3	83.55	
Solids	47.2	21.79	100	35.8	18.82	100	34.3	16.45	100
Inorganic matter	3.10	1.43	6.56	3.04	1.06	8.50	3.36	1.61	9.81
Proteins	34.4	15.88	73.56	25.9	13.63	72.44	24.5	11.73	71.40
Lipins	9.7	4.33	19.88	6.8	3.59	19.06	6.5	3.11	18.89

And, from the above figures, the proportions of the chemical changes through the fasting are calculated as follows :—

TABLE III.

	Initial values	Gains and losses after fasting		Their percentageous values		Percentage of the finishes for the initials	
		in the light	in the darkness	in the light	in the darkness	in the light	in the darkness
Live weight	216.5	−26.4	− 7.9	−14	− 4	86	96
Water	169.3	−15.0	+ 5.0	− 9	+ 2	91	102
Solids	47.2	−11.4	−12.9	−24	−27	76	73
Inorganic matter	3.10	− 0.06	+ 0.29	− 8	+ 8	92	108
Proteins	34.4	− 8.5	− 9.9	−25	−28	75	73
Lipins	9.7	− 2.9	− 3.2	−30	−33	70	67

Proportions of proteins and lipins in the solids consumed are shown in the next table.

TABLE IV.

	In the light		In the darkness	
Solids	11.4	100	12.9	100
Proteins	8.5	74	9.9	75
Lipins	2.9	25	3.2	25

Conclusion.

From the above mentioned results, effects of the light on fasting young salmons at just absorbed their york-sacs are as follows:—

1. There occour loss of live weight and consumption of solids (proteins and lipins) in the light and in the darkness, the percentageous values of solids (organic) are less in the light than in the darkness whilst inorganic matter accumulated, on the contrary, in the latter condition.

On fasting, percentageous amount of water increase, but notwithstanding solids decrease. It is very interesting phenomenon that live weights of the fasting fishes in the darkness are heavier than those in the light but solids of the formers less than the latters because the formers absorb some water from the surrounding medium during fasting.

2. Percentageous amounts of proteins and lipins in the solids consumed are the same in the both sides but those to the initial values (before the experiments) are greater in the darkness than in the light.

3. Inorganic contents in percentage increase through the fasting becassue the consumption of inorganic matter is lower than the others.

This result may be assertained the result in the preceeding report.

ON THE DETERMINATION AND PREPARATION OF CYSTINE.

By

Y. Okuda and T. Kobayashi.

(Received Oct., 8th., 1929).

Determination

Several years ago, one of the writers established a method of determining cystine in proteins and named it the Iodine method[1]. The principle of this method is to decolorize the hydrolysate with animal charcoal and to reduce cystine entirely into cysteine with zinc powder, and then to titrate the colorless solution with a standard iodate solution in the presence of iodide and hydrochloric acid, until the yellow color due to free iodine remains for one

minute.

Teruuchi and Okabe[2] described the iodine methode as an accurate and simpler than any other known and recommendable method, declaring also that it seems to be an important invention in the quantitative estimation of cystine. But they attempted to modify the method by omitting the use of animal charcoal, and by employing starch solution as in ordinary iodometry.

In this present investigation we have compared the original and the modified methods and found that the original is more reliable. The reasons for this conclusion are as follows:—

1. It is a well known fact that cystine is adsorbed by charcoal, but it is readily redissolved by washing.

2. By omitting the use of charcoal the reduction of cystine into cystein by zinc dust is sometimes incomplete.

To verify the fact some comparative studies of these two methods were carried out, with gelatine cleavage products, adding a definite quantity of cystine. On the determination by the original method, we recovered 99.3～99.9 per cent of added cystine, while by the modified method the results were as low as 87.8～89.5 per cent.

The cystine contents of egg albumine, edestine, human hair, fibrin, casein and legumin as determined by the two methods were nearly equal respectively, although a little less in the case of the medified method. But great difference were observed in the cystine content of muscle proteins obtained by the two methods, it being very small in the case of the modified method.

From these results it seems that there are some substances, in the hydrolysate of proteins especially such as gelatine and muscle proteins, which cover the surface of zinc particles by a union like adsorption and retard the reduction, but that these substances are removed by the use of charcoal.

Preparation.

Some comparative studies on the methods of preparing cystine, such as those of Abderhalden, Okabe, Folin, Harris, and Schmidt, were performed, and Okabe's and Abderhalden's were found better than the others.

But the so-called isoelectric points of both cystine and tyrosine being very near, the first crystal of cystine obtained by the methods of Okabe and Abderhalden usually contains very much tyrosine. It is the case especially by Okabe's method.

In studying the solubility of tyrosine, at the isoelectric point of cystine, in the presence of several salts, we found that the presence of a certain quantity of ammonium acetate retarded the crystallization of tyrosine but had no influence on cystine.

The basis of the present method of preparing cystine depends upon the use of ammonium acetate.

Procedure:— Take 100 g. of hair in a flask of 1 litre capacity, add 300 c.c. of 25 per cent hydrochloric acid, and heat on a water bath, under a reverted condenser until it is dissolved, and then boil on a sand bath for 8 hours. Add about 10 g. of animal charcoal to the hydrolyzed solution, heat for 20 minutes, filter, and wash the charcoal well. Evaporate the filtrate and the the washings under a diminished pressure into a thick syrup to remove most of the hydrochloric acid. Dissolve the syrup with boiling water and make it up into about 100 c.c., and immediately add some crystals of ammonium acetate, keeping the solution always warm. Use congo red paper as an outside indicator, and when the solution ceases to give a blue coloration to the paper, add more crystals of ammonium acetate until the solution contains about 30 per cent of the salt. Heat the solution to dissolve the salt and then allow the solution to stand in a cold place for 24 hours, stirring from time to time, to get the first crystals of cystine. (The reaction of the solution in this case is usually pH 4.0～4.4)

The first crystals give generally, in the case of hair, no Millon's reaction, and the yield is about 70 per cent of its cystine content as determined by the iodine method. As the crystals are somewhat colored, they should be once recrystallized to get pure crystals.

For this purpose, dissolve the crude crystals in dilute hydrochloric acid solution, decolorize the solution with animal charcoal, and then evaporate it into about 100 c.c. Add ammonium acetate in cystals until the hydrogen ion concentration of the solution is about pH 4.5, using brom cresol green as the indicator. Keep the solution in a cold place for 24 hours.

References.

(1) Okuda: J. Depart. Agr. Kyushu Imp. Uni., **1**, 163, (1925).
(2) Teruuchi & Okuda: J. of Biochemistry. **8**, 459, (1928).

STUDIES ON VITAMIN C.

I. ON THE OCCURRENCE OF VITAMIN C IN CELERY.

By

TOMIJI MATSUOKA.

(From the Laboratory of Nutritional Chemistry, Department of Agriculture, Kyoto Imperial University.)

(Received Oct. 11 th., 1929).

The celery plant belongs to the umbelliferae (Apium Graveoleus L.). It has a peculiar flavour and taste, and its inner petioles are blanched and eaten as a salad, while both the blades and petioles are dried and used for the flavouring of soup, stew etc, and for the preparation of powder celery and celery salt. According to T. Onda, celery was first imported into our country in 1978. In that time it was used among a few people who were fond of it ; consequently, its cultivation was of a small extent. At present, however, there has been a steady increase in its production especially during the last 5 years. At this juncture it may be rather necessary to investigate the nutritive values of celery vegetables.

In 1917, McCollum demonstrated that celery contains vitamin A and in 1922 Osborne and Mendel showed that it contains a comparatively fair amount of vitamin B, but there are yet no studies on vitamin C contained in celery. Therefore, the writer tried to make some experiments on this problem and their results are as follows :—

Experimental.

1. Animals on the experiments and the basal rations.

(a) Animals on the experiments.

In the animal test of the experiments guinea pigs were used for the detection of vitamin C content in celery. The first thing needed for my experiments was to get the animals of exactly the same stock, that is, those descended from the same ancestors. I obtained a pair of guinea pigs having white hair and red eye from a commercial animal house, and made them increase by means of incest breeding.

They were fed with the basel ration and water, and weighed at least once every five days.

(b) The basal ration.

The basal ration used on the experiments consisted of the following :—

Wheat bran	47
Oat powder	47
Goat milk	20
$CaCO_3$	3
NaCl	3

The oat used in the basal ration was ground in a meal crusher and the resulting meal was carefully sifted so that it might be free both from large particles and the skin. The goat milk was heated in an electric heater at 120°C for two hours.

By the results of the feeding it is evident that the heating destroyed nearly all vitamin C in the milk, while vitamin A and vitamin B in the basal ration were preserved enough for the normal growth of guinea pigs.

2. *Feeding experiments on the basal rations.*

The writer's experiments consisted of three processes, (a), (b), (c), for ascertaining whether the basal ration will be fit for the determination of vitamin C.

(a) Feeding on the basal ration alone.

All pigs shwed indications of the prevalence of scurvy and died after being fed for 17 to 30 days.

(b) The therapeutic tests.

By the above results it is evident that when we feed the pigs on the basal ration alone, they all without exception are inflicted with scurvy. Then the writer tried with experiments to know whether the basal ration plus vitamin C might have a therapeutic value or not. First, as the source of vitamin C he gave to the pigs 10 c.c. per day of radish juice, which was poured into their mouths by force. The writer must make an apology here that several pigs died during the first few experiments owing to his unskilfulness in the therapeutic treatments. The pigs had a normal appetite during the first period of the test, but afterwards showed the first signs of scurvy on the 19 th. to 20 th. day. After having been on the ration for 22 to 28 days 10 c.c. of radish juice per day were added. It was chloroformed at the end of 80 days after its health was in a normal condition.

Post mortem examination showed that the pig's health had been entirely restored to normal condition by means of this new ration.

(c) Feeding on the basal ration plus vitamin C.

As the source of vitamin C the writer used 10 c.c. of radish juice or 10 grammes of radish per day.

Fed with this ration, all animals were of an exceptionally fair appearance and showed every indication of being in excellent health. According to the above results it is clear that the basal ration is deficient for the feeding of guinea pigs, but in adding a certain quantity of vitamin C to it, it will be a

perfect food for feeding guinea pigs.

3. Feeding on celery experiment.

The celery used on the experiments was of the Golden Self Blanching variety cultivated in 1928 on the farm of this laboratory. It was well washed with tap water and dried in the shade, after wards being preserved in a cool place.

(a) Expriments supplied with 1.0 to 2.0 grammes of celery per day per 100 grammes of body weight.

The pigs normally consumed the diet all showing an increase of body weight. As the animals were of an exceptionally fair appearance and showed indications of being in excellent health, they were chloroformed after having been on this diet for 50 to 60 days. The post mortem examination showed that the animals carried through a normal growth on this diet. From the results obtained it is evident that there is a fairly rich content of vitamin C in the stalk and leaf of the celery cultivated in Japan and that the supply of 1.0 to 2.0 grammes of celery per day 100 grammes of body weight is enough to prevent guinea pigs from being afflicted with scurvy.

(b) Experiments supplied with 0.75 grammes of celery per day per 100 grammes of body weight.

Most of the animals feeding on it could not escape from being afflicted with scurvy and died in 20 to 45 days of feeding ; namely, four animals out of six gave indications of scurvy on 15 to 32 days of feeding and afterwards died, while the other two lived keeping a normal appearance.

Post mortem examination of the latter (after being chloroformed) showed that they had been in a nearly normal state of health.

(c) Experiments supplied with 0.5 grammes of celery per day per 100 grammes of body weight.

All animals could not escape from being afflicted with scurvy on this diet, and afterwards all died in the sickness.

According to the above results of feeding on the diet with 0.5 grammes of celery per day per 100 grammes of body weight as the source of vitamin C, the animals could not escape from being afflicted with scurvy and gave symptoms of every kind of the sickness and died, all of them, after having been fed on the diet for 18 to 24 days. From the above results on the celery experiments, vitamin C content in 0.5 to 0.75 grammes of celery per day is not enough to prevent the pigs fed on the ration from the symptoms of scurvy but the content of 1.0 to 2.0 grammes of celery per day per 100 grammes of body weight is enough to keep them in normal growth. It is also evident from these results that vitamin C content of celery is nearly equal to that of orange or of lemon juice and cabbage, and that it is superior

to that of germinated barley or peas or lentiles. (about two times.)

Summary.

1. The basal ration used by the writer was only lacking in vitamin C, which being added afterwards, it became a perfect diet for the feeding of guinea pigs.

2. An amount of 1.0 to 2.0 grammes of celery per day per 100 grammes of body weight is enough for preventing the animals from being afflicted with scurvy; namely vitamin C content of the celery cultivated in Japan in equal to that of lemon or orange or radish juice.

STUDIES ON THE CASTOR-BEAN LIPASE. V.

THE ACTION OF ULTRA-VIOLET RAYS UPON THE VEGETABLE OILS, FROM THE VIEWPOINT OF ENZYMOLOGY

By

Etsuo Takamiya.

(Received Oct, 15th., 1929).

In this study, the action of ultra-violet rays upon vegetable oils was investigated, the changes in enzymic hydrolysis-velocity of oils caused by irradiation being the criterion.

The fact that irradiated oils, as well as those that are ozonised, are less readily hydrolysed by the enzyme than the original oils, and the further fact that the physico-chemical changes of oils caused by irradiation with ultra-violet rays are quite similar as those caused by the action of ozone, led us to the conclution, that it is due to the action of ozone produced during the use of a mercury vapour quartz lamp that the irradiated oils are less readily hydrolysed by the enzyme than the original oils. Moreover, a portion of cottonseed oil was irradiated with ultra-violet rays, eliminating by a certain method the action of ozone produced during the irradiation, and another portion of the cottonseed oil was treated with irradiation combined with the action of the ozone. In the former case there was no change in the enzymic hydrolysis-velocity but in the latter case it decreased. Viewed in the light of enzymology, the action of ultra-violet rays upon the vegetable oils must, therefore, be no more than the action of ozone, and the ultra-violet rays correspond to an ozone-generator.

STUDIES ON THE VITAMIN D.

By

Etsuo Takamiya.

(Received Oct, 13th., 1929).

I. The Actions of Ozone and Ultra-Violet Rays Upon the Cholesterol and Oils.

I have reported in this journal that the action of ultra-violet rays upon vegetable oils viewed in the light of enzymology, must be no more than the action of ozone, and the ultra-violet rays correspond to an ozone-generator. Consequently the effects of ozone and ultra-violet rays upon cholesterol and oils were compared in this study, by means of the criteria of photoactivity, colour reactions etc., and it was confirmed that the action of ozone is closely analogous to that of ultra-violet rays. If the several phenomena caused by irradiation with ultra-violet rays in photoactivity, colour reactions etc. would stand in a relation to the photochemical conversion of pro-vitamin into vitamin D, it would be readily seen that ergosterol could be converted into vitamin D by the action of ozone, without the irradiation with ultra-violet rays. And if ergosterol would not become an antirachitic active substance by the action of ozone, it would be seen that the above-mentioned phenomena would not have relation to the photochemical conversion of ergosterol into vitamin D, and it would be pseudo-phenomena caused by the accessory action of ultra-violet rays.

In the extent of my experiment, the same or closely analogous results were obtained by the irradiation of ultra-violet rays and by the action of ozone. Consequently it will be suggested that a correlation such as exists between vitamin D and ultra-violet rays will also exist between vitamin D and ozone.

II. Rickets and Ozone.

It was generally admitted fact that pro-vitamin is converted photochemically into vitamin D by the irradiation with ultra-violet rays, and it has also recently been discovered that ergosterol is the pro-vitamin. Studies on vitamin D, therefore, are now advanced from the relation "pro-vitamin + ultra-violet rays = vitamin D" to the relation of "ergosterol + ultra-violet rays = vitamin D".

Nevertheless, the significance of the action of ultra-violet rays on the photochemical conversion into vitamin D still remains unexplained. If this

problem could be solved, the chemical nature of vitamin D would be elucidated.

I reported in the preceding paper that a correlation such as exists between vitamin D and ultra-violet rays will also exist between vitamin D and ozone.

In the present investigation, consequently, the author attempted the feeding expriments to ascertain whether ozone has an antirachitic potency or not. From the results of the feeding experiments, it became clear that the albino rats, which became rachitic by the feeding with the rachitogenic diet of McCollum Diet No. 3143, are healed of their rickets by means of ozonized air. Consequently, it will be not difficult to establish the following relation :—

Ergosterol (known) + Ozone (known) = Vitamin D.

The author reports here only a fact that ozone has the power to heal reckets of rats. The problems such as the correlation of ergosterol and ozone, and its mechanism etc. will be discussed in the next occasion. The problem as between the photochemical conversion of pro-vitamin into vitamin D, and the process of oxidation, was discussed in detail in the original paper.

DETERMINATION OF HYDROGEN ION CONCENTRATION OF SOILS BY THE QUINHYDRONE METHOD.

I. ON SOME FACTORS INFLUENCING THE pH VALUES.

By

A. Itano, S. Arakawa and A. Matsuura.

(Received Nov. 2nd., 1929).

Preliminary to the determination of hydrogen ion concentration of soils representing the dry-farm and rice-field, collected from different parts of Japan through the courtesy of Agricultural Experiment Stations, it was felt necessary to formulate the method of treatment of the samples, since various investigators have treated the samples differently with somewhat varied results. The following factors were investigated and the results are given below :—

(1) N/10 calomel, simplified-saturated calomel and the standard quinhydrone electrodes all give the similar results.

(2) An amount of quinhydrone to be used for each determination is 0.05 g. per 15 c.c. of the sample.

(3) No change of E. M. F. is observed within five minutes after the equilibrium is reached.

(4) By air-drying the soil from the rice-field, the pH is increased or becomes alkaline.

(5) No appreciable influence is observed by different degree of grinding the soil.

(6) The suspension gives a smaller pH value than the filtrate.

(7) Shaking the soil with water for five minutes is sufficient to get the result.

(8) The best soil-water ratio is 1 : 1, and 1 : 1.5 ratio is prefered for some soils, such as humic and volcanic.

From the results noted above, the following procedure is adopted for our investigation :

A definite amount or air-dried soil sample is placed in Erlenmyer flask and the neutral distilled water is added, 1 : 1 (1 : 1.5 is used in case of humic or volcanic soil.), and rubber stoppered. The flask is shaken for five minutes, filtered through a neutral filter paper. Fifteen cubic centimeter of the filtrate is taken and 0.05 g. of quinhydrone is added and the determination is made as usual.

STUDIES ON THE FERMENTATION PRODUCTS BY MOULD FUNGI. VI.

ASPERGILLUS GLAUCUS. PART II.

By

Yusuke Sumiki.

(Agricultural Chemical Laboratory, Tokyo Imperial University).

(Received Nov. 22 nd , 1929).

Aspergillus glaucus is cultured in the following media at 30°C consisting of glucose (10%), K-mono & diphosphate (each 0.015%), Mg-sulphate & Ca-chloride (each 0.01 %), dist. water (90 %), Fe-chloride & Na-chloride (each trace), Ca-carbonate and nitrogen source.

No.	Total (l.)	Day	N-source (%)	Ca-carbonate (%)
23	8	76	peptone 1	0.3

24	8	33	urea	0.1	0.3
25	7.3	37	peptone	0.1	1
26	9	31	〃	0.1	—

1. *Isolation and identification of produced gluconic acid.*

The fermented medium is evapolated to small bulk, acidified with sulphuric acid and filtered. The filtrate is extracted with ether. The ether insoluble part is boiled with the excess of Ca-carbonate for neutralization, and filtered after colling. The filtrate is evapolated under reduced pressure and leaved alone in the ice box. Ca-gluconate crystallized out is filtered, dried at 100°C (yield : 465 g. from No. 23, 660 g. from No.s 24 & 25, 330 g. from No. 26) and recrystallized from boiling water twice. This crystal is dissoived again in water and added with the slightly excess of theoretical amount of oxalic acid. After filtration to remove Ca-oxalate, the filtrate is extracted with ether. One part of this ether insoluble fraction is evapolated on the water bath, leaved alone over the conc. sulphuric acid and gluconic acid is obtained as lactone melting at 128~32°C.

Another part of ether insoluble fraction is treated with the HCl-salt of phenylhydrazine and Na-acetate. Then phenylhydrazide crystallizes out which melts at 198-200°C after recrystallization from hot water 2 times.

Subst. 0.0646 g. 5.4 ccm. N (16.5°, 764.0 m.m.).
$C_{12}H_{18}O_6N_2$ Cal. N=9.79%
Fou. N=9.90%

2. *Quantitative determination of gluconic acid.*

The constitution of medium is the same as above described but nitrogen source. The quantity of glucose contained in the medium is given as 7.5% by the method of Bertrand in spite of preparation to contain 10% of glucose. 400 ccm. of such medium are taken with 25 g. of Ca-carbonate in one culture flask and at each test one flask is put to use. So the yield described in the table shows the theoretical % of Ca-gluconate from 400 ccm. of medium, i. e. from 30 g. of glucose.

The method of determination is as follows. The clutured medium is filtered and the mycelium and Ca-carbonate are washed with hot water. The filtrate is evapolated, acidified with sulphuric acid and extracted with ether. The ether insoluble part is added with baryta water to neutrallize only sulphuric acid using congo red paper as indicator and without filtration is added with the excess of Ca-carbonate and boiled. After cooling and filtering, the filtrate is evapolated to a small quantity, added with 2 volumes of alcohol (75%) and leaved alone in the ice box. Ca-gluconate crystallized out is filtered, washed with dil. alcohol, dried at 100°C and weighed as the crystal containing one molecule of crystal water.

(1) Yields from various concentrations of different nitrogen sources.

N-source	% of N-source	Day	Temp. (C°)	Ca-gluconate (g.) (with 1 H_2O)	Yield (theoretical %)
Peptone	1.5	24	30	12.0	32.2
		34	"	11.0	29.7
		50	"	6.5	17.6
	0.7	15	"	14.0	37.8
		30	"	13.0	35.2
		50	"	7.0	18.9
	0.2	15	"	8.0	21.6
		30	"	17.5	47.3
		40	"	15.5	41.9
	0.1	15	"	14.0	37.8
		30	"	27.0	73.0
		50	"	26.5	71.6
	0.05	15	"	3.5	9.5
		30	"	16.5	44.6
		40	"	18.5	50.0
Am.-sulphate	1.5	14	"	0.5	1.3
		30	"	18.5	50.0
		40	"	21.5	58.1
	0.7	14	"	1.5	4.1
		30	"	8.5	23.0
		40	"	1.0	2.7
	0.1	15	"	2.0	5.4
		30	"	8.5	23.0
		40	"	4.0	10.8
Urea	1.5	15	"	0	0
		40	"	4.5	12.1
	0.7	15	"	3.0	8.1
		30	"	12.5	34.0
	0.1	15	"	12.0	32.4
		30	"	15.0	40.5
		40	"	14.5	39.1
Na-nitrate	1.5	15	"	1.0	2.7
		30	"	7.0	18.9
	0.7	15	"	1.0	2.7
		30	"	7.0	18.9
	0.1	15	"	3.0	8.1
		30	"	12.0	32.2
		40	"	22.0	59.4

(2) Yields from various temperatures.

From the above table, it is acertained that 0.1% of peptone is the best nitrogen source and the best concentration. Next table shows the yields from various temperatures when 0.1% of peptone is used as nitrogen source.

Temp.(C°)	Day	Ca-gluconate (g.) (with 1H_2O)	Yield (%) (theoretical)
30	15	14.0	37.8
	30	27.0	73.0
	50	26.5	71.6
25	15	1.0	2.7
	30	17.5	47.3
	40	12.0	32.2

below 20	15	2.0	5.4
	30	9.0	24.2
	40	12.0	32.2

(3) Yields from various concentration of glucose.

Merck's glucose is used (its purity : 98.98%) and the quantity of glucose which is consumed is determined by the method of Bertrand. 400 ccm. of medium are taken in one culture flusk, so 10% solution of glucose in one culture flusk corresponds to 39.50 g. of glucose, 20% to 79.18 g. 30% to 118.77 g.

Glucose (%)	Peptone (%)	Day	Temp. (C°)	Ca-Gluconate (g) (with $1H_2O$)	Yield (theoretical%) from glucose used as medium.	Yield (theoretical%) from glucose consumed.
10	0.1	30	30	35.5	72.0	82.9
20	0.1	〃	〃	47.5	48.2	82.0
20	0.2	〃	〃	34.0	34.5	57.8
30	0.1	〃	〃	10.0	6.8	
30	0.3	〃	〃	10.0	6.8	

3. *Conclusion.*

(1) By Asp. glaucus, gluconic acid is produced from glucose used as carbon source and gluconic acid is identified as its lactone and phenylhydrazide.

(2) 0.1% of peptone as the nitogen source, 10% of glucose as the carbon source and cultivation during 30 days at 30°C are the optimum conditions to produce gluconic acid.

(3) Under this optimum condition, the yield of gluconic acid from glucose used as the medium is 72.0% of the theory which corresponds to 82.9 % of the theory from glucose consumed by the fungi.

STUDIES ON THE FERMENTATION PRODUCTS BY MOULD FUNGI. VII.

PENICILLIUM OLIVACEUM.

By

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1. *Condition of cultivation.*

Penicillium olivaceum is cultured in the following media at 30°C during

29～126 days.

Glucose 10%, Pepton 0.2%, K_2HPO_4 & KH_2PO_4 each 0.015%, $MgSO_4.7H_2O$ & $CaCl_2$ each 0.01%, NaCl & $FeCl_3$ each trace, dist. Water 90%, $CaCO_3$.

No.	Pepton (%)	Total (l.)	Day	
1	0.7	8	120	for qualitative determination.
2	0.2	3	39	
3	1.0	6	29	
4	0.05	4.5	90	
5	0	4	126	
6	2.0	6	100	
7	0.1	9	90	
8	0	1.6	15～90	for quantitative determination. 400 ccm. of medium are taken with 15 g. of $CaCO_3$ in one culture flusk and one of these flusks is put to use at each test.
9	0.05	〃	〃	
10	0.1	〃	〃	
11	0.5	〃	〃	
12	1.0	〃	〃	
13	2.0	〃	〃	

2. *Isolation and identification of fermentation products.*

(1) By steem distillation, the volatile substances in the fermented medium are isolated from the non-volatile substances. This distillate gives no reactions of acid and of alcohol, so it is acertained alcohol and volatile acid are not produced. The residue by steem distillation, is evapolated, acidified with sulphuric acid and extracted with ether. From the ethereal solution, organic acids are obtained and their Ca-salts are prepared to isolate each acid. From the ether insoluble part, gluconic acid is obtained. (see author's report, Bull. Agri. Chem. Soc., Japan, **4**, 13, 1928).

(2) The fermented medium is filtered and the filtrate, if it gives acid reaction, is neutrallized with Ca-hydroxide accurately. The ppt. is the Ca-oxalate (ppt. A). When the filtrate from the Ca-salt of oxalic acid is evapolated to small bulk, the Ca-salt of citric acide crystallizes out (ppt. B). After filtration while it is hot, the filtrate is added with the excess of alcohol to precipitate aliphatic acids as their Ca-salts. These salts are acidified with suphuric acid, filtered and extracted with ether. The ether insoluble part is neutrallized with the excess of $CaCO_3$ by boiling. After cooling and filtering, the filtrate is evapolated, added with alcohol and gluconic acid is precipitated as its Ca-salt (ppt. C).

The mycelium and the ppts. isolated by the first filtration of fermented medium are macerated with dil. HCl in the boiling water bath. The macerated solution is neutrallized roughly with $CaCO_3$, precisely with $Ca(OH)_2$. Then only oxalic acid is ppt. ed as its Ca-salt (ppt. D). The filtrate from this Ca-oxalate, is evapolated, put together with ppt. B and dissolved in water. From this solution, citric acid is ppt.ed as its Pb-salt by adding Pb-acetate and the free citric acid is obtained by treating with H_2S.

Ppt.s A and D are put together, added with sulphuric acid, filtered and extracted with ether. From this ethereal solution, free oxalic acid is obtained.

(a) Citric acid.

The crystal recrystallized from boilling water and dried in the desiccator over sulphuric acied, melts at 153~6°.

Subst. 0.1188 g. CO_2 0.1600 g. H_2O 0.0478 g.
$C_6H_8O_7$ Cal. C=37.50%, H=4.17%.
Fou. C=36.73%, H=4.47%.
0.2122 g. of subst. requires 33.4 ccm. of NaOH.
33.4 ccm. of NaOH=32.8823 ccm of N/10 H_2SO_4.
M.W.=65×3=195 (cal. 192),

(b) Oxalic acid.

The crystal recrystallized from hot water, melts at 100° alone or in admixture with authentic oxalic acid.

Subst 0.5384 g. AgCl 0.5026 g.
$Ag_2C_2O_4$ Cal. Ag=71.05%.
Fou. Ag=70.26%.

(c) Gluconic acid.

The crude Ca-salt of gluconic acid (ppt. C), is recrystallized from boiling water twice, added with the theoretical quantity of oxalic acid and filtered. By treating with the HCl-salt of phenylhydrazine and Na-acetate, the filtrate produces phenylhydrazide of gluconic acid. After recrystallization from boiling water, hydrazide melts at 198°.

Subst. 0.0682 g. N 5.7 ccm (18.0°, 762.0 mm).
$C_{12}H_{18}O_6N_2$ Cal. N=9.79%.
Fou. N=9.82%.

3. *Quantitative determination of fermented products.*

The condition of cultivation is already described on section 1. The method of quantitative determination is as follows. By filtration, the fermented medium is separated in 2 parts, the mycelium & the ppt. and the filtrate. The filtrate is acidified with sulphuric acid, evapolated, filtered and extracted with ether. From ethereal solution, one part of citric acid (A) is obtained. The ether insoluble solution is neutrallized primarily with Ba-hydroxide using congored paper as indicator only sulphuric acid, secondarily with Ca-carbonate boiling them, filtered, evapolated and again filtered. The filtrate is added with 2 volumes of alcohol (80%). The produced ppt. of Ca-gluconate, is filtered, dried at 100° and weighed as the crystal with one molecule of crystal water.

The mycelium and the ppt. are macerated completely in the boiling water bath with dil. HCl and the macerated solution is neutrallized roughly with Ca-carbonate, then accurately with Ca-hydroxide and filtered. The ppt. is dried at 100° and weighed as the Ca-oxalate. The filtrate is evapolated, acidified with sulphuric acid and extracted with ether. From ethereal solution

the remaining part of citric acid (B) is weighed.

In the following table, the yield from 400 ccm. of medium is shown.

No	Day	Mycellium (g.)	Citric acid (g.)			Oxalic acid (g.)		Gluconic acid (g)	
			A	B	Total	Salt	Free	Salt	Free
8	15	0.15	0.25	0.05	0.30	0.15	0.10	1.3	1.13
9	〃	0.75	0.1	0.05	0.15	〃	〃	1.95	1.71
10	〃	1 7	0.3	〃	0.35	〃	〃	2.0	1.75
11	〃	3.6	0.8	0.3	1.1	0.2	0.14	3.4	2.98
12	〃	8.6	〃	0.4	1.2	0.25	0.17	3.6	3.15
13	〃	10.4	〃	0.3	1.1	〃	〃	5.4	4.73
8	30	0.15	0.3	0.05	0.35	0.15	0.10	1.35	1.13
9	〃	0.65	0.4	〃	0.45	〃	〃	1.9	1.68
10	〃	1.4	0.3	1.05	1.35	〃	〃	2.1	1.83
11	〃	5.2	0.7	0.5	1.2	0.25	0.17	3.4	2.98
12	〃	8.9	0.4	〃	0.9	0.5	0.35	3.05	2.67
13	〃	12.7	0.55	0.15	0.7	〃	〃	4.7	4.11
8	50	0.15	0.3	0.05	0.305	0.15	0.10	2.0	1.75
9	〃	1.0	〃	0.5	0.8	〃	〃	2.9	2.54
10	〃	1.25	0.35	0.45	〃	0.25	0.17	〃	〃
11	〃	7.3	0.7	1.15	1.85	0.35	0.24	3.0	2.63
12	〃	8.1	0.3	0.6	0.9	0.6	0.41	1.9	1.68
13	〃	9.3	0.45	0.2	0.65	1.0	0.69	4.1	3.59
8	90	0.15	0.45	0.05	0.50	0.15	0.10	2.0	1.75
9	〃	1.2	0.3	0.65	0.95	〃	〃	2.5	2.19
10	〃	1.9	0.3	〃	〃	0.3	0.21	2.3	2.01
11	〃	7.1	0.35	0.75	1.10	0.45	0.31	3.0	2.63
12	〃	9.1	0.3	1.0	1.3	0.4	0.28	4.2	3.68
13	〃	12.55	0.35	1.1	1.45	0.5	0.35	5.2	4.56

4. *Conclusion.*

(1) The fermentation products from glucose by Penicillium olivaceum is studied.

(2) As the fermentation products, citric, gluconic and oxalic acids are isolated and identified.

(3) The yield of each acid from the medium containing 0～2% of peptone is also determined quantitatively.

(4) The volatile acid and alcohol are not produced.

ON THE NATURAL PIGMENTS OF RAW SILK FIBRE OF THE DOMESTIC COCOON. (PART I).

XANTHOPHYLL OF THE YELLOW COCOON.

By

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(Received Dec. 3rd., 1929).

There are many kinds of the domestic cocoon differently colored, viz. white, yellow, green, orange, red and etc. and the raw silk reeled from them is also differed in their coloration according to the pigment of the cocoon.

In this research the most popular yellow cocoons are taken into account and I have succeeded to isolate this unknowm pigment in its purest form and identified it as xanthophyll.

Experimental Results.

Yellow cocoon layers of Kokusan Ô-7-Gô are digested with 80% alcohol under 15 lbs. pressure for half an hour. After the extract is evaporated under reduced pressure, the yellow pigments are taken up by ether and then saponified with methyl-alcoholic potash. After well washing with water, the ether solution is evaporated to dryness and the residue is dissolved in light petroleum ether and 85% methyl alcohol. Almost all the yellow pigments go into methyl alcohol by successive extraction. Transferring the pigments into ether from methyl alcohol, ether is evaporated and the crude pigments can be obtained by recrystallization from boiling methyl alcohol. After removal of the waxy substance and non-crystallizable pigments by boiling light petroleum ether, the pure pigment crystallizes out as quadratic tablet showing some indentations by recrystallization from methyl alcohol.

The color of the crystal is pleochromatic purple red by reflected light and shows steel-blue reflection when suspended in the solvent but the color transmitted is yellow to orange.

This pure pigment shows many characteristic features or plant xanthophyll as follows.

(1) Solvent :— it crystallizes out with one molecule of solvent, entirely insoluble in boiling light petroleum ether, sparingly soluble in cold alcohol and carbon-disulphide but soluble in ether, chloroform and acetone.

(2) Melting point :— 174°～175° (uncorr.)

(3) Elementary composition :—

C% 84.32, H% 10.24, O% 5.44.

(4) Molecular weight :— 552.6 g.

(5) Absorption band :—

Solvent	Alcohol	Carbon-disulphide
Band 1	487～472 (μμ)	516～500 (μμ)
〃 2	454～439 (〃)	480～468 (〃)
〃 3	421～414 (〃)	447～ (〃)

(6) Iodide can be easily obtained by adding iodine to the ether solution as dark violet tufte.

(7) Adsorption by calcium carbonate :— it is relatively stronger adsorbed by dried calcium carbonate from its carbon disulphide or petroleum ether solution than carotin.

(8) Color reaction :— it shows many color reactions relating carotinoids and vitamin-A.

(9) Colloidal solution of the digment is easily obtained by dilution with water the concentrated alcoholic solution of the pigment.

All the above mentioned properties point to that of xanthophyll of plant origin.

The yellow pigment of Kokusan Si-7-Gô (so-called Kinkô) cocoon layers is also isolated in its pure form by the same process and conditions and identified it as xanthophyll, too. All the properties show quite good coincidence with that of the former, eg.,

Melting point	174°～175° (uncorr.)
Molecular weight	556.3 g.
Elementary composion	C% 84.06, H% 10.10, O% 5.84.

The content of xanthophyll in the cocoon layer of both species was estimated colorimetricall and found that the former contains 2.39 mg. and the latter 2.21 mg. per 10 g. of air dried cocoon layers.

Finally, the following items are discussed.

(1) Xanthophyll of the raw silk fibre is found in physicochemical combination with sericin.

(2) The yellow pigments mainly consist of pure xanthophyll and its oxidation products.

(3) Xanthophyll of the cocoon is derived biologically from that of mulberry leaves.